CHROMATOGRAM

Retention time: 15 (aspartic acid), 20 (glutamic acid), 27.5 (asparagine), 30 (glutamine), 31.5 (serine), 32.5 (homoserine), 35.5 (glycine), 40 (taurine), 42 (alanine), 44 (β-alanine), 52 (norvaline), 54 (valine), 60 (leucine)

KEY WORDS

derivatization

REFERENCE

Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE

Matrix: solutions

Sample preparation: Mix 5 μL of a 0.05-10 mM solution with 20 μL 100 mM NaOH and 150 μL 5 mM 4-(N-phthalimidyl)benzenesulfonyl chloride in acetone, heat at 50° for 15 min, dilute 10-fold with mobile phase, inject a 20 μL aliquot. (Synthesis of 4-(N-phthalimidyl)benzenesulfonyl chloride (Phisyl-Cl) is as follows. Mix 2.68 g o-phthalaldehyde in 100 mL diethyl ether with 1.86 g aniline in 20 mL diethyl ether, stir at room temperature overnight, filter. Wash the solid with diethyl ether and recrystallize it from MeOH to yield N-phenylphthalimidine. Drop 6.6 g chlorosulfonic acid onto 2.09 g of crystals of N-phenylphthalimidine in an ice bath with vigorous stirring over 20 min, heat at 60° for 2 h, add 30 g crushed ice, recrystallize the precipitate from benzene to obtain 4-(N-phthalimidyl)benzenesulfonyl chloride as fine colorless needles (mp 186-187°) (Caution! Benzene is a carcinogen!).)

HPLC VARIABLES

Guard column: 25 × 4.6 5 μm ODS (Yamamura, Japan)

Column: 250 × 4.6 5 μm YMC AM-303 ODS (Yamamura)

Mobile phase: Gradient. A was MeCN:30 mM pH 6.5 Tris buffer 10:90. B was MeCN:30 mM pH 6.5 Tris buffer 75:25. A:B 100:0 for 8 min, 90:10 for 12 min, 80:20 for 18 min, 70:30 for 10 min, 55:45 for 5 min, 40:60 for 7 min, 0:100 for 5 min, re-equilibrate at initial conditions for 15 min (step gradient).

Flow rate: 0.6

Injection volume: 20

Detector: F ex 295 em 425

CHROMATOGRAM

Retention time: 18.5 (cysteine), 19 (aspartic acid), 20 (glutamic acid), 30 (hydroxyproline), 32.5 (asparagine), 35 (serine), 36 (methionine), 36.5 (threonine), 38 (glycine), 39 (alanine), 43 (proline), 47 (valine), 51 (isoleucine), 52.5 (leucine), 55 (phenylalanine), 56.5 (cystine), 59.8 (ornithine), 60.2 (lysine), 61 (histidine), 63 (tyrosine)

Limit of detection: <0.2 pmole

KEY WORDS

derivatization

REFERENCE

Tsuruta,Y.; Date,Y.; Kohashi,K. Phthalimidylbenzenesulfonyl chlorides as fluorescence labeling reagents for amino acids in high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *502*, 178–183.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:50 mM NaOH 80:20 with 30 mg reagent, after 2 min elute with 500 μL MeCN, add 500 μL water to the eluate,

mix, inject a 20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb CN 100
Mobile phase: MeCN:water:trifluoroacetic acid 20:80:0.1
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: 2 (valine), 2.2 (methionine), 3.3 (phenylalanine), 4 (tryptophan)

KEY WORDS

derivatization

REFERENCE

Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE

Matrix: solutions
Sample preparation: Mix 200 μL of a 10 mM solution of amino acid in 100 mM sodium bicarbonate with 200 μL 10 mM Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) in acetone (freshly prepared), heat at 40° with frequent mixing for 1 h, cool, add 100 μL 200 mM HCl, degas, filter, inject an aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm μBondapak
Mobile phase: Gradient. X was MeCN containing 0.1% trifluoroacetic acid. Y was water containing 0.1% trifluoroacetic acid. X:Y from 10:90 to 60:40 over 50 min (mobile phase A) or from 5:95 to 20:80 over 70 min (Mobile Phase B) or isocratic MeCN:20 mM pH 4.0 sodium acetate buffer 8:92
Flow rate: 2
Detector: UV 340

CHROMATOGRAM

Retention time: k' 2.96 (L-His) (Mobile Phase A), k' 1.80 (D-His) (Mobile Phase A), k' 4.18 (L-Asp) (Mobile Phase A), k' 4.66 (D-Asp) (Mobile Phase A), k' 4.80 (L-Thr) (Mobile Phase A), k' 6.11 (D-Thr) (Mobile Phase A), k' 5.70 (L-Glu) (Mobile Phase A), k' 6.37 (D-Glu) (Mobile Phase A), k' 6.73 (L-Ala) (Mobile Phase A), k' 7.72 (D-Ala) (Mobile Phase A), k' 7.55 (L-Val) (Mobile Phase A), k' 9.08 (D-Val) (Mobile Phase A), k' 7.95 (L-Leu-NH2) (Mobile Phase A), k' 9.73 (D-Leu-NH2) (Mobile Phase A), k' 8.14 (L-Tyr) (Mobile Phase A), k'

9.05 (D-Tyr) (Mobile Phase A), k' 8.12 (L-Trp-NH2) (Mobile Phase A), k' 9.40 (D-Trp-NH2) (Mobile Phase A), k' 8.17 (L-Met) (Mobile Phase A), k' 9.85 (D-Met) (Mobile Phase A), k' 8.92 (L-Phe-NH2) (Mobile Phase A), k' 10.57 (D-Phe-NH2) (Mobile Phase A), k' 9.02 (L-Leu) (Mobile Phase A), k' 10.65 (D-Leu) (Mobile Phase A), k' 9.51 (L-Ile) (Mobile Phase A), k' 11.45 (D-Ile) (Mobile Phase A), k' 9.83 (L-Trp) (Mobile Phase A), k' 11.25 (D-Trp) (Mobile Phase A), k' 9.96 (L-Nle) (Mobile Phase A), k' 11.87 (D-Nle) (Mobile Phase A), k' 10.18 (L-allylglycine) (Mobile Phase A), k' 11.69 (D-allylglycine) (Mobile Phase A), k' 10.44 (L-Cys (tert-butyl)) (Mobile Phase A), k' 12.01 (D-Cys (tert-butyl)) (Mobile Phase A), k' 10.80 (L-Cystine) (Mobile Phase A), k' 11.47 (D-Cystine) (Mobile Phase A), k' 11.04 (L-Phe) (Mobile Phase A), k' 12.40 (D-Phe) (Mobile Phase A), k' 11.89 (L-Lys (bis derivative)) (Mobile Phase A), k' 12.58 (D-Lys (bis derivative)) (Mobile Phase A), k' 13.54 (L-His (triphenylmethyl)) (Mobile Phase A), k' 13.89 (D-His (triphenylmethyl)) (Mobile Phase A), k' 16.29 (L-Cys (triphenylmethyl)) (Mobile Phase A), k' 17.21 (D-Cys (triphenylmethyl)) (Mobile Phase A), k' 17.80 (L-Ser) (Mobile Phase B), k' 16.63 (D-Ser) (Mobile Phase B), k' 20.48 (L-Cys (S-acetamidomethyl)) (Mobile Phase B), k' 22.67 (D-Cys (S-acetamidomethyl)) (Mobile Phase B), k' 25.08 (L-Pro) (Mobile Phase B), k' 26.68 (D-Pro) (Mobile Phase B), k' 26.2 (L-Asn) (Mobile Phase C), k' 44.7 (D-Asn) (Mobile Phase C), k' 26.6 (L-Gln) (Mobile Phase C), k' 45.7 (D-Gln) (Mobile Phase C), k' 51.7 (L-Arg) (Mobile Phase C), k' 103.3 (D-Arg) (Mobile Phase C)

KEY WORDS
chiral; derivatization

REFERENCE
Adamson,J.G.; Hoang,T.; Crivici,A.; Lajoie,G.A. Use of Marfey's reagent to quantitate racemization upon anchoring of amino acids to solid supports for peptide synthesis, *Anal.Biochem.*, **1992**, *202*, 210–214.

SAMPLE
Matrix: solutions
Sample preparation: Mix 70 μL of an aqueous amino acid solution with 300 μL phthalaldehyde solution and 30 μL thiol solution for 2 min, inject an aliquot. (Prepare the phthalaldehyde solution by dissolving 60 mg o-phthalaldehyde in 3 mL MeOH and 15 mL 400 mM pH 9.4 sodium borate buffer. Prepare the thiol solution by dissolving 6.5 mg D-S-acetyl-3-mercapto-2-methylpropionic acid (Novabiochem) in 1 mL 1 M NaOH, stir at room temperature for 10 min, adjust the pH to 7.0 with phosphoric acid. The solution contains D-3-mercapto-2-methylpropionic acid.)

HPLC VARIABLES
Column: 250 × 4 3 μm Nucleosil-120-C18
Mobile phase: Gradient. MeCN:50 mM pH 6.0 sodium acetate buffer 0:100 for 10 min, to 62.5:37.5 (?) over 100 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 338 em 415 (long-pass filter)

CHROMATOGRAM
Retention time: 21 (D-Asp), 25 (L-Asp), 28.5 (D-Glu), 32 (L-Glu), 33 (D-Asn), 34 (D-Ser), 36 (L-Asn), 37 (L-Ser), 37.5 (D-Gln), 41 (D-Thr), 42 (L-Gln), 42.5 (Gly), 43 (D-His), 43.5 (L-His), 45 (D-Arg), 46 (L-Thr), 48 (D-Ala), 49 (L-Arg), 51.5 (L-Ala), 54 (D-Tyr), 57 (L-Tyr), 61 (D-Val), 64 (D-Met), 68 (L-Met), 69 (D-Ile, L-Val), 70 (D-Trp), 71 (D-Phe), 72 (D-Leu), 74 (L-Trp), 75 (L-Phe), 78.5 (L-Ile), 80 (L-Leu), 91 (D-Lys), 93 (L-Lys)
Limit of detection: 2 pmole

KEY WORDS
derivatization; comparison with other thiols; chiral

REFERENCE

Duchateau,A.L.L.; Knuts,H.; Boesten,J.M.M.; Guns,J.J. Enantioseparation of amino compounds by derivatization with o-phthalaldehyde and D-3-mercapto-2-methylpropionic acid, *J.Chromatogr.*, **1992**, *623*, 237–245.

SAMPLE

Matrix: solutions

Sample preparation: Mix 10 μL of a 100 μM solution in 200 mM pH 9.3 borate buffer containing 4 mM disodium EDTA with 100 μL 50 mM DBD-F in MeCN, heat at 60° for 30 min, add 890 μL MeOH:acetic acid 99:1, inject a 10 μL aliquot. (Synthesis of DBD-F is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products. It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Sumichiral OA 2500(S) ((S)-1-naphthylglycyl-3,5-dinitrophenylamide silica gel) (Sumika Analytical, Osaka)

Mobile phase: MeOH containing 20 mM ammonium acetate

Flow rate: 1

Injection volume: 10

Detector: F ex 450 em 590

CHROMATOGRAM

Retention time: 13 (D-Leu), 15 (L-Leu)

KEY WORDS
derivatization; chiral; comparison with other derivatizing reagents

REFERENCE
Imai,K.; Fukushima,T. Derivatization with fluorogenic benzofurazan reagents of amino acid enantiomers and their separation on a Pirkle type column, *Biomed.Chromatogr.*, **1993**, *7*, 275–276.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL of a 100 μM solution in 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA with 30 μL 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole in MeCN, heat at 60° for 2 min, add 960 μL MeOH:acetic acid 99:1, dilute 5 times with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumichiral OA 2500(S) ((S)-1-naphthylglycyl-3,5-dinitrophenylamide silica gel) (Sumika Analytical, Osaka)
Mobile phase: MeOH containing 20 mM ammonium acetate
Flow rate: 1
Injection volume: 10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 18.57 (D-Leu), 20.8 (L-Leu), 25.3 (D-Phe), 30.6 (L-Phe)
Limit of detection: 25-34 fmole

KEY WORDS
derivatization; chiral

REFERENCE
Imai,K.; Fukushima,T.; Uzu,S. Sensitive determination of enantiomers of amino acids derivatized with the fluorogenic reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole, separated on a Pirkle-type column, Sumichiral OA 2500(S), *Biomed.Chromatogr.*, **1993**, *7*, 177–178.

SAMPLE
Matrix: solutions
Sample preparation: 32 μL Amino acid solution in 50 mM pH 9.0 borate buffer + 69 μL 1.96 mM cyanide, mix, add 6 μL 2.25 mM naphthalene dicarboxaldehyde mixed with 13 μL 50 mM pH 9.0 borate buffer, mix, let stand for 30 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 MicroPak SP C18
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 10:90. B was MeCN:MeOH:THF:50 mM pH 6.8 potassium phosphate buffer 55:10:3.5:31.5. A:B from 90:10 to 45:55 over 45 min, to 20:80 over 1 min, maintain at 20:80 for 3 min, return to initial conditions over 2 min, re-equilibrate for 3 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: 7 (aspartic acid), 10 (glutamic acid), 18 (histidine), 20 (serine), 22 (arginine), 22.5 (glycine), 24 (threonine), 26 (alanine), 29 (tyrosine), 31 (α-aminobutyric acid), 35.5 (valine), 36 (methionine), 40.5 (isoleucine), 41 (phenylalanine), 42 (leucine)
Limit of detection: 200 fmole

KEY WORDS
derivatization; discussion of matrix interference in paper

REFERENCE
Lai,F.; Sheehan,T. Matrix effects in the derivatization of amino acids with naphthalene dicarboxaldehyde, 9-fluorenylmethyl chloroformate and phenylisothiocyanate, *BioTechniques*, **1993**, *14*, 642–649.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 μL of a solution of amino acids in 50 mM pH 9.3 borate buffer containing 1 mM disodium EDTA with 250 μL 1 mM DIFOX in MeCN, let stand in the dark at room temperature for 1 h, add 500 μL MeCN:1 M HCl 50:50, inject an aliquot. (Prepare DIFOX as follows. Gently reflux 21 g benzoin and 45 g urethane (Caution! Urethane is a carcinogen!) in 300 mL DMF for 6 h, cool, pour into water, filter, recrystallize to give 4,5-diphenyl-2-oxazolone (mp 211°), treat with phosphorus oxychloride to give 2-chloro-4,5-diphenyloxazole (Ber. 1956, 89, 1749). Add 3 g anhydrous KF to 1.62 g 2-chloro-4,5-diphenyloxazole in 60 mL MeCN, add 1.5 g 18-crown-6/MeCN complex, reflux for 24 h, cool, filter. Concentrate the filtrate and add 50 mL hexane, stir for 10 min, filter rapidly, repeat the extraction. Combine the filtrates and concentrate them to give a yellow oil, distil to give DIFOX (2-fluoro-4,5-diphenyloxazole) (bp 130°/0.02 mm Hg) (Analyst 1993, 118, 257). Prepare 18-crown-6/MeCN complex by heating 50 g 18-crown-6 in 125 mL MeCN until a homogeneous solution is obtained (use a calcium sulfate drying tube), stir the solution vigorously as it cools to room temperature, cool in a dry ice/acetone bath, filter rapidly, dry under high vacuum at ≤40° over 2-3 h to give 18-crown-6/MeCN complex (mp 36.5-38°) (Caution! The complex is hygroscopic!) (J. Org. Chem. 1974, 39, 2445). No experimental details are given for the phosphorus oxychloride reaction above but a procedure for the p-N,N-dimethylaminosulfonyl analogue proceeds as follows. Suspend 2 g dried 4,5-bis(p-N,N-dimethylaminosulfonylphenyl)-2-oxazolone in 30 mL phosphorus oxychloride, stir at 0°, add 610 μL triethylamine dropwise, heat at 100° for 7 h, remove the excess phosphorus oxychloride on a rotary evaporator. Dissolve the residue in dichloromethane and wash with cold saturated sodium bicarbonate, dry the organic layer over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on silica gel to give 2-chloro-4,5-bis(p-N,N-dimethylaminosulfonylphenyl)oxazole (Analyst 1993, 118, 257).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-8 (Supelco)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 phosphate buffer 25:75. B was MeCN: 50 mM pH 7.0 phosphate buffer 50:50. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 320 em 420

CHROMATOGRAM
Retention time: 6 (aspartic acid), 6.5 (glutamic acid), 9.5 (hydroxyproline), 13.5 (arginine), 15 (proline), 15.5 (glycine), 16 (alanine), 17.5 (tyrosine), 20 (valine), 20.5 (methionine), 21.5 (leucine, isoleucine), 23.5 (tryptophan, phenylalanine), 35 (lysine)
Limit of detection: 19-64 fmole

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Chokshi,H.P.; Givens,R.S.; Carlson,R.G.; Lunte,S.M.; Kuwana,T. Fluorescence and chemiluminescence detection of oxazole-labelled amines and thiols, *Biomed.Chromatogr.*, **1993**, *7*, 208–216.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL 4 mM amino acids in water with 100 μL buffer, 50 μL 50 mg/mL 1-thio-β-D-galactopyranose sodium salt in water, and 50 μL 40 mg/mL o-

phthalaldehyde in MeOH, stir thoroughly for 1 min, inject a 10 μL aliquot. (Buffer was 500 mg boric acid in 19 mL water, pH adjusted to 9.30 with 45% KOH.)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8
Mobile phase: Gradient. A was 50 mM sodium acetate adjusted to pH 6.10 with acetic acid. B was MeOH:100 mM pH 7.60 sodium acetate buffer 90:10. A:B 100:0 for 8 min, to 45:55 over 47 min, to 0:100 over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 10
Detector: F ex 360 em 420

CHROMATOGRAM

Retention time: 4.6 (L-Asp, D-Asp), 8.9 (L-Glu, D-Glu), 12.1 (L-Ser), 15.2 (D-Ser), 20.7 (L-Tre), 24.2 (D-Tre), 27.7 (L-Ala), 29.9 (L-Arg), 30.1 (D-Ala), 30.3 (D-Arg), 37.1 (L-Tyr), 37.8 (D-Tyr), 43.0 (D-Val), 44.9 (L-norvaline), 45.1 (L-Trp), 45.4 (L-Val), 45.9 (D-norvaline), 46.8 (D-Trp), 47.6 (L-Phe), 49.6 (D-Phe), 51.8 (L-Leu), 51.8 (D-Ile), 52.2 (L-norleucine), 52.2 (L-Ile), 53.1 (D-norleucine), 53.1 (D-Leu), 54.2 (D-Lys), 55.5 (L-Lys)
Limit of detection: <10 pmole

KEY WORDS

derivatization; chiral

REFERENCE

Jegorov,A.; Triska,J.; Trnka,T. 1-Thio-β-D-galactose as a chiral derivatization agent for the resolution of D,L-amino acid enantiomers, *J.Chromatogr.A*, **1994**, *673*, 286–290.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate a solution of amino acids in 10 mM HCl under a stream of nitrogen at 50°, add 50 μL MeCN, evaporate under a stream of nitrogen, add 50 μL MeCN:MeOH:triethylamine 10:5:2, sonicate for 1 min, add 3 μL butyl isothiocyanate, cap the vial with a septum, heat at 40° for 30 min. Pass nitrogen into the vial with one needle and apply a vacuum with another needle until solvent is removed (ca. 15 min), add 100 μL MeCN, remove MeCN in a similar fashion. Dissolve the residue in 500 μL 200 mM ammonium acetate, filter (0.20 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid. B was MeCN:50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid 50:50. C was MeCN:water 70:30. A:B:C from 100:0:0 to 85:15:0 over 8 min, to 70:20:10 over 6 min, to 60:20:20 over 6 min, to 20:0:80 over 5 min, to 0:0:100 over 5 min, maintain at 0:0:100 for 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 250

CHROMATOGRAM

Retention time: 8.05 (Asp), 9.00 (Glu), 11.27 (Hyp), 13.75 (Asn), 13.75 (Ser), 14.39 (Gly), 14.63 (Gln), 15.71 (His), 16.13 (Thr), 16.29 (Ala), 16.45 (Arg), 16.84 (Pro), 17.76 (Cyt), 20.27 (Tyr), 20.51 (Val), 21.73 (Met), 23.29 (Ile), 23.67 (Leu), 25.18 (Phe), 25.53 (Trp), 26.16 (Lys), 26.84 (Cys)
Limit of quantitation: 0.5 nmole

KEY WORDS
Asp and Ser not resolved; derivatization

REFERENCE
Woo,K.-L.; Lee,S.-H. Determination of protein amino acids as butylthiocarbamyl derivatives by reversed-phase high-performance liquid chromatography with precolumn derivatization and UV detection, *J.Chromatogr.A*, **1994**, *667*, 105–111.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of an amino acid solution with 100 μL 500 mM pH 9.0 sodium borate buffer and 250 μL 5 mM 4-phenylazobenzyloxycarbonyl chloride in MeCN, let stand for 5 min, add 250 μL 40 mM 1-aminoadamantane in acetone:water 75:25, mix, let stand for 5 min. Remove an 80 μL aliquot and add it to 320 μL MeCN:500 mM pH 4.0 sodium acetate buffer 50:50, inject a 20 μL aliquot. (4-Phenylazobenzyloxycarbonyl chloride can be purchased from Bachem, Bubendorf, Switzerland. Synthesis is as follows. Dissolve 10 g 4-nitrobenzylalcohol in 100 mL MeOH, add 1 mL triethylamine, add 2 g Raney nickel, hydrogenate at room temperature and atmospheric pressure, filter. Evaporate the filtrate to dryness, add benzene (Caution! Benzene is a carcinogen!), evaporate to dryness to remove water, repeat this step to obtain 4-aminobenzyl alcohol as crystals. Mix 7.7 g 4-aminobenzyl alcohol with a solution of 7.4 g nitrosobenzene in 40 mL acetic acid with stirring at 0°, after 3 h filter, wash the solid with dilute acetic acid to obtain 4-phenylazobenzylalcohol (mp 142.5-143°). Dilute the filtrate with a lot of water, filter, extract with hot carbon tetrachloride, crystallize from carbon tetrachloride to obtain more p-phenylazobenzyl alcohol. Dissolve 10.9 g phosgene in 40 mL dioxane, add 5 g p-phenylazobenzylalcohol at 0°, stir at 0° for 15 min, let stand at room temperature for 3 h, filter, evaporate the filtrate to dryness under reduced pressure. Recrystallize the residue from petroleum ether to give 4-phenylazobenzyloxycarbonyl (mp 82-83°) (Helv. Chim. Acta 1958, 41, 491).)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: Gradient. MeCN:100 mM pH 7.0 sodium acetate from 22:78 to 50:50 over 40 min, to 80:20 over 5 min, to 100:0 over 1 min, maintain at 100:0 for 9 min, re-equilibrate at initial conditions for 12 min.
Column temperature: 45
Flow rate: 1.25
Injection volume: 20
Detector: UV 320

CHROMATOGRAM
Retention time: 7 (Asp), 8 (Glu), 14 (Ser), 15.5 (Gly), 16 (Thr), 16.5 (Pro, Arg), 17 (Ala), 19 (Tyr (mono-derivative)), 22 (Val), 22.5 (Met), 25 (Ile), 25.5 (Leu), 26.5 (Phe), 31.5 (cystine), 42.5 (His), 43 (Lys), 47 (Tyr (bis-derivative))
Limit of detection: 1-10 pmole

KEY WORDS
derivatization; comparison with other derivatizing reagents

REFERENCE
Brückner,H.; Lüpke,M. Use of chromogenic and fluorescent oxycarbonyl chlorides as reagents for amino acid analysis by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 295–307.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of an amino acid solution with 100 μL 400 mM pH 8.0 sodium borate buffer and 100 μL 3 mM 9-fluorenylmethyl chloroformate in acetone

(MeCN if UV detection is used), let stand for 2 min, add 100 μL 40 mM 1-aminoadamantane in acetone:water 75:25, let stand for 2 min. Remove an 20 μL aliquot and add it to 380 μL MeCN:500 mM pH 4.0 sodium acetate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-8
Column: 250 × 4 4 μm Superspher 60 RP-8
Mobile phase: Gradient. A was THF:DMF:100 mM pH 4.6 sodium acetate buffer 5:5:90. B was MeCN. A:B from 93:7 to 85:15 over 10 min, to 50:50 over 25 min, to 0:100 over 5 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 10 min. (Reflux DMF in the presence of ninhydrin then distil.)
Column temperature: 45
Flow rate: 1.25
Injection volume: 20
Detector: F ex 263 em 313

CHROMATOGRAM

Retention time: 8 (CyA), 15.2 (Asn), 16 (Gln), 17 (Asp), 17.5 (Ser), 18.5 (Glu), 19.5 (Thr), 20 (Arg), 20.5 (Gly), 21 (homo-Arg), 23 (Ala), 24 (Tyr, mono-derivative), 25 (Pro), 26 (Met), 28 (Val, GABA), 29 (Phe), 30.8 (Ile), 32 (Leu), 32.5 (Hyl), 34 (His), 35 (Orn), 36.5 (Lys), 38 (Tyr, bis-derivative)
Limit of detection: 5-150 fmole

KEY WORDS

derivatization; comparison with other derivatizing reagents

REFERENCE

Brückner,H.; Lüpke,M. Use of chromogenic and fluorescent oxycarbonyl chlorides as reagents for amino acid analysis by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 295–307.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Amino acid solution in 500 mM pH 8 sodium borate buffer + 50 μL 20 mM norvaline in 500 mM pH 8 sodium borate buffer + 200 μL 30 mM 9-fluorenylmethyl chloroformate in dry acetone, shake, allow to stand at room temperature for 10 min, add 200 μL 25 mM 1-aminoamantadine in MeOH, let stand for 2 min, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Aminotag (Varian)
Mobile phase: Gradient. A was MeCN:isopropanol 90:10. B was THF:50 mM sodium acetate buffer 4:96, adjusted to pH 4.03 with glacial acetic acid. A:B from 20:80 to 25:75 over 25 min, to 30:70 over 10 min, to 40:60 over 10 min, to 50:50 over 10 min, to 80:20 over 15 min, to 100:0 over 5 min, re-equilibrate at initial conditions for 15 min.
Column temperature: 32
Flow rate: 1.5
Injection volume: 20
Detector: UV 265 or F ex 265 em 340

CHROMATOGRAM

Retention time: 12 (arginine), 15 (taurine), 16 (serine), 17 (aspartic acid), 20 (glutamic acid), 21 (threonine), 22 (glycine), 31 (proline), 37 (alanine), 41.5 (methionine), 43.5 (valine), 48 (phenylalanine), 48.5 (tryptophan), 49 (isoleucine), 50 (leucine), 59 (histidine), 60.5 (lysine), 64 (tyrosine)
Internal standard: norvaline (45)

KEY WORDS

derivatization

REFERENCE
Carratù,B.; Boniglia,C.; Bellomonte,G. Optimization of the determination of amino acids in parenteral solutions by high-performance liquid chromatography with precolumn derivatization using 9-fluorenylmethyl chloroformate, *J.Chromatogr.A*, **1995**, *708*, 203–208.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 20 mM Amino acid (10 mM for Lys) in MeCN:water 50:50 + 2 μL triethylamine + 20 μL 40 mg/mL O-tetraacetyl-β-D-glucopyranosyl isothiocyanate in MeCN, let stand at room temperature for 30 min, add 50 μL 1 M HCl, add 830 μL MeCN:water 25:75, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 7 μm Hypercarb S
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN:water 90:10. A:B 70:30 for 15 min, to 64:36 over 10 min, maintain at 64:36 for 3 min, to 61:39 over 5 min, maintain at 61:39 for 2 min, to 55:45 over 10 min, maintain at 55:45 for 5 min, to 0:100 over 33 min, maintain at 0:100 for 10 min, return to initial conditions over 3 min.
Flow rate: 1.1
Injection volume: 5-20
Detector: UV 250

CHROMATOGRAM
Retention time: k' 7.15 (D-His), k' 4.38 (L-His), k' 10.54 (D-Arg), k' 8.92 (L-Arg), k' 11.89 (D-Ser), k' 11.60 (L-Ser), k' 12.43 (D-Pro), k' 12.78 (L-Pro), k' 16.67 (D-Thr), k' 13.71 (L-Thr), k' 17.07 (D-Ala), k' 14.49 (L-Ala), k' 17.97 (D-Asp), k' 16.03 (L-Asp), k' 17.97 (D-Glu), k' 16.03 (L-Glu), k' 25.52 (D-Val), k' 21.69 (L-Val), k' 28.02 (D-Leu), k' 25.17 (L-Leu), k' 29.9 (D-Ile), k' 27.41 (L-Ile), k' 41.0 (D-Phe), k' 34.27 (L-Phe), k' 37.17 (D-Lys), k' 36.65 (L-Lys), k' 45.04 (D-Tyr), k' 34.67 (L-Tyr)

KEY WORDS
chiral; derivatization

REFERENCE
Chan,W.C.; Micklewright,R.; Barrett,D.A. Porous graphitic carbon for the chromatographic separation of O-tetraacetyl-β-D-glucopyranosyl isothiocyanate-derivatised amino acid enantiomers, *J.Chromatogr.A*, **1995**, *697*, 213–217.

SAMPLE
Matrix: solutions
Sample preparation: Shake a slurry of 100 μmoles amino acid, 100 μmole 3,5-dinitrobenzoyl chloride, and 300 μmoles propylene oxide (Caution! Propylene oxide is a carcinogen!) in 5 mL dry THF was shaken at room temperature for 7 days, filter, evaporate to dryness under reduced pressure, dissolve the residue in 30 mL MeOH, add Amberlite IR-120, reflux for 1 h, evaporate to dryness, reconstitute with dichloromethane, chromatograph on a small column of Kieselgel 60 (Merck) with hexane:ethyl acetate 90:10, evaporate to dryness, inject an aliquot of a solution in the mobile phase.

HPLC VARIABLES
Column: 300 × 4 CSP 1 chiral column (Details of column preparation are in paper.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 6.26 (first enantiomer, α = 1.18 (alanine)), k' 4.48 (first enantiomer, α = 1.16 (2-aminobutyric acid)), k' 3.65 (first enantiomer, α = 1.11 (norvaline)), k' 3.21 (first enantiomer, α = 1.14 (valine)), k' 3.26 (first enantiomer, α = 1.08 (norleucine)), k' 3.07 (first enantiomer, α = 1.09 (leucine)), k' 2.84 (first enantiomer, α = 1.09 (isoleucine)), k' 6.27 (first enantiomer, α = 1.02 (phenylglycine)), k' 5.53 (first enantiomer, α = 1.11 (phenylalanine)), k' 7.11 (first enantiomer, α = 1.16 (methionine)), k' 5.61 (first enantiomer, α = 1.00 (proline)), k' 3.27 (first enantiomer, α = 1.02 (N-methylalanine))

KEY WORDS

chiral; derivatization

REFERENCE

Chen,C.-C.; Lin,C.-E. HPLC separation of enantiomers of amino acids and amino alcohols on ionically bonded chiral stationary phases consisting of cyanuric chloride with amino acid and dialkylamine substituents, *J.Chromatogr.Sci.*, **1995**, *33*, 229–235.

SAMPLE

Matrix: solutions

Sample preparation: 400 μL Amino acid solution + 100 μL 1 M pH 8.9 borate buffer + 500 μL 10 mM 2-(9-anthryl)ethyl chloroformate (Eka Nobel) in MeCN, mix, let stand for 5 min, add 1 mL pentane, extract, discard the pentane layer, inject an aliquot of the reaction mixture.

HPLC VARIABLES

Column: 650 × 0.25 5 μm Kromasil C8 (Eka Nobel)

Mobile phase: Gradient. MeCN:100 mM pH 4.1 acetate buffer from 40:60 to 85:15 over 45 min

Flow rate: 0.005-0.01

Injection volume: 0.06-1

Detector: F ex 351 (16 mW Ar laser) em 412 (bandpass filter) or UV 256

CHROMATOGRAM

Retention time: 8.5 (Arg), 11 (Asp, Ser), 12 (Glu), 13 (Thr), 15 (Gly), 17.5 (Ala), 20.5 (Pro), 22 (Met), 24 (Phe), 24.5 (Val), 25.5 (Cys), 26.5 (Cystine), 27.5 (Ile), 28 (Leu), 33.5 (His), 36 (Lys), 39 (Tyr)

Limit of detection: 400 nM (UV), 0.30 nM (F)

KEY WORDS

capillary HPLC; comparison with capillary electrophoresis; derivatization

REFERENCE

Engström,A.; Andersson,P.E.; Josefsson,B.; Pfeffer,W.D. Determination of 2-(9-anthryl)ethyl chloroformate-labeled amino acids by capillary electrophoresis and liquid chromatography with absorbance or fluorescence detection, *Anal.Chem.*, **1995**, *67*, 3018–3022.

SAMPLE

Matrix: solutions

Sample preparation: Mix Vortex 10 μL peptide solution in pyridine:water 50:50 and 10 μL 20 mM reagent in pyridine:water 50:50, heat at 50° for 15 min, wash 3 times with 100 μL porions of n-heptane:dichloromethane 90:10. Remove the aqueous phase and dry it in a centrifugal evaporator at 50° for 15 min, add 30 μL trifluoroacetic acid to the residue, heat at 50° for 10 min, evaporate to dryness under a stream of nitrogen, reconstitute with 20 μL water, extract 3 times with n-heptane:dichloromethane 80:20 (retain the aqueous phase for the next cycle). Combine the extracts and evaporate them to dryness under a stream of nitrogen, reconstitute in MeCN, inject an aliquot. (Perform all reactions under nitrogen. N-Terminal amino acid is derivatized and sequenced. Reagent

was 7-[(N,N- dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate and it was prepared as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm ,Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Stir 50 mg DBD-F in 15 mL MeCN and add 100 μL 28% ammonia in water, stir at room temperature overnight, evaporate under reduced pressure, recrystallize from MeCN to give 4-amino-7-N,N-dimethylaminosulfonyl-2,1,3-benzoxadiazole as pale yellow needles (DBD-NH2) (mp 214-7°). Add 1 mL 30% thiophosgene in benzene dropwise to 200 mg DBD-NH2 in 15 mL MeCN, reflux for 5 h, evaporate under reduced pressure, extract the residue twice with 20 mL portions of chloroform. Filter the extracts and evaporate them to dryness, dissolve the residue in chloroform and chromatograph on 15 g silica gel (G-200) with chloroform. Evaporate the eluate to dryness and recrystallize 7-[(N,N-dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate from benzene-n-hexane to give yellow-white crystals (mp 122-4°) (Biomed.Chromatogr. 1992, 7, 56).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC J'sphere ODS H-80 (YMC) + 250 × 4.6 5 μm YMC-Pack Ph phenyl (YMC) in series
Mobile phase: MeCN:water 60:40 containing 10 mM formic acid
Flow rate: 0.5
Injection volume: 20
Detector: F ex 387 em 524

CHROMATOGRAM

Retention time: 14 (Ser), 17 (Arg), 17.5 (Thr), 21 (Gly), 23 (Tyr), 24 (Ala), 26 (Asn), 28 (His), 29 (Pro), 30.5 (Asp), 31 (Glu), 32 (Met), 33, 45 (Lys), 36 (Val), 41 (Phe), 46 (Ile), 48 (Leu)
Limit of detection: 200 pmole

KEY WORDS
derivatization

REFERENCE
Matsunaga,H.; Santa,T.; Hagiwara,K.; Homma,H.; Imai,K.; Uzu,S.; Nakashima,K.; Akiyama,S. Development of an efficient amino acid sequencing method using fluorescent Edman reagent 7-[(N,N-dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate, *Anal.Chem.*, **1995**, *67*, 4276–4282.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL of an amino acid solution in MeCN:water:triethylamine 50:50:2 with 10 μL 5 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, heat at 55° for 10 min, add 480 μL 1 M acetic acid in MeCN:water 50:50, dilute 10-fold with MeCN, inject a 5 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei (TCI America, Portland OR). Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro- 2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°) (Analyst 1995, 120, 385).

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 25:75:0.05 (A) or 30:70:0.05 (B) or 35:65:0.05 (C) or 40:60:0.05 (D) or 45:55:0.05 (E)
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: F ex 490 em 530

CHROMATOGRAM
Retention time: k' 10.71 (D-alanine (A)), k' 11.19 (L-alanine (A)), k' 3.34 (D-cystine (E)), k' 4.03 (L-cystine (E)), k' 10.94 (D-isoleucine (C)), k' 12.30 (L-isoleucine (C)), k' 5.61 (D-

leucine (D)), k' 6.29 (L-leucine (D)), k' 7.91 (D-lysine (D)), k' 9.13 (L-lysine (D)), k' 6.37 (D-methionine (C)), k' 6.92 (L-methionine (C)), k' 12.17 (D-norleucine (C)), k' 13.75 (L-norleucine (C)), k' 4.87 (D-proline (B)), k' 6.06 (L-proline (B)), k' 12.52 (D-phenylalanine (C)), k' 14.90 (L-phenylalanine (C)), k' 7.77 (D-threonine (A)), k' 8.25 (L-threonine (A)), k' 5.95 (D-tryptophan (D)), k' 7.05 (L-tryptophan (D)), k' 9.20 (D-tyrosine (B)), k' 10.00 (L-tyrosine (B)), k' 6.11 (D-valine (C)), k' 6.81 (L-valine (C))

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Liu,Y.-M. High-performance liquid chromatographic resolution of amino acid enantiomers derivatized with fluorescent chiral Edman reagents, *J.Chromatogr.A*, **1995**, *689*, 23–30.

SAMPLE
Matrix: solutions
Sample preparation: 35 μL Solution of amino acids in water + 35 μL 3.3 mg/mL o-phthalaldehyde in MeOH:water 50:50 + 35 μL 4 mg/mL N-acetyl-L-cysteine in MeOH:water 50:50 + 175 μL 400 mM pH 9.4 potassium borate buffer, mix well, let stand at room temperature for at least 2 min (J.Chromatogr. 1989, 471, 263), neutralize with trifluoroacetic acid, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil 120-C18
Mobile phase: MeOH:buffer 40:60 (Buffer 50 mM ammonium acetate adjusted to pH 6.0 with acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: MS, Finnigan MAT TSQ 70 triple quadrupole, thermospray, first and second quadrupoles in rf-only mode, third quadrupole in scan and mass selective mode, the column effluent was mixed with 1% trifluoroacetic acid in water pumped at 0.25 mL/min, first 5 mL of column effluent was diverted from the detector, source 200°, repeller 120 V, vaporizer 90

KEY WORDS
derivatization; chiral

REFERENCE
van Leuken,R.G.J.; Duchateau,A.L.L.; Kwakkenbos,G.T.C. Thermospray liquid chromatography/mass spectrometry study of diastereomeric isoindole derivatives of amino acids and amino acid amides, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1459–1464.

SAMPLE
Matrix: solutions
Sample preparation: 30 μL 100 mM Amino acid in 1 M HCl + 45 μL 1 M sodium bicarbonate + 500 μL 10 mM reagent in DMSO, heat at 100° for 1 h, add 1.425 mL DMSO, inject a 1-5 μL aliquot. (Prepare reagent as follows. Add 2.14 g L-valinamide hydrochloride in 10 mL water to 2.58 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 35 mL acetone with stirring while maintaining the temperature at 5-10°, add 8 mL 2 M sodium carbonate, stir at 20° for 1 h, evaporate to dryness under reduced pressure, take up the residue in 350 mL ethyl acetate, wash with two 175 mL portions of water, add 10 mL toluene, evaporate to dryness to give N-(4,6-dichloro-[1,3,5]triazin-2-yl)-L-valine amide as a white solid (mp 151-152°). Add 1.64 g L-phenylalaninamide in 10 mL water to 2.64 g N-(4,6-dichloro-[1,3,5]triazin-2- yl)-L-valine amide in 35 mL acetone with stirring at room temperature, add 10 mL 1 M sodium bicarbonate, stir at room temperature for 3 h, evaporate to dryness under reduced pressure, take up the residue in 350 mL ethyl acetate, wash with two 175 mL portions of water, add 10 mL toluene, evaporate to dryness to give

N-[4-((S)-1-carbamoyl-2-methylpropylamino)-6-chloro-[1,3,5]triazin-2-yl]-L-phenylalanine amide (mp 128-130°).)

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil 100 C18
Mobile phase: MeCN:10 mM pH 4 sodium acetate 20:80
Flow rate: 1
Injection volume: 1-5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.90 (L-Glu), 5.89 (D-Glu), 8.26 (L-Pro), 12.76 (D-Pro), 38.28 (L-Phe), 78.14 (D-Phe)

KEY WORDS
derivatization; chiral

REFERENCE
Brückner,H.; Wachsmann,M. Liquid chromatographic separation of amino acid enantiomers on a silica-bonded chiral *s*-triazine column, *J.Chromatogr.A*, **1996**, *728*, 447–454.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 40 μL aliquot of a solution of amino acids in 100 mM HCl with 100 μL 500 mM pH 9.0 potassium borate buffer and 250 μL 0.5 mM 4-phenylazobenzyloxycarbonyl chloride in MeCN, let stand for 5 min, add 100 μL reagent, mix, let stand for 5 min. Remove an 80 μL aliquot and add it to 320 μL MeCN:500 mM pH 4.0 sodium acetate buffer 20:80, mix, inject a 20 μL aliquot. (Prepare reagent by mixing 3 mL n-heptylamine, 15 mL MeCN, and 175 mL 100 mM HCl, pH 7-8. 4-Phenylazobenzyloxycarbonyl chloride can be purchased from Bachem, Bubendorf, Switzerland. Synthesis is as follows. Dissolve 10 g 4-nitrobenzylalcohol in 100 mL MeOH, add 1 mL triethylamine, add 2 g Raney nickel, hydrogenate at room temperature and atmospheric pressure, filter. Evaporate the filtrate to dryness, add benzene (Caution! Benzene is a carcinogen!), evaporate to dryness to remove water, repeat this step to obtain 4-aminobenzyl alcohol as crystals. Mix 7.7 g 4-aminobenzyl alcohol with a solution of 7.4 g nitrosobenzene in 40 mL acetic acid with stirring at 0°, after 3 h filter, wash the solid with dilute acetic acid to obtain 4-phenylazobenzylalcohol (mp 142.5-143°). Dilute the filtrate with a lot of water, filter, extract with hot carbon tetrachloride, crystallize from carbon tetrachloride to obtain more p-phenylazobenzyl alcohol. Dissolve 10.9 g phosgene in 40 mL dioxane, add 5 g p-phenylazobenzylalcohol at 0°, stir at 0° for 15 min, let stand at room temperature for 3 h, filter, evaporate the filtrate to dryness under reduced pressure. Recrystallize the residue from petroleum ether to give 4-phenylazobenzyloxycarbonyl chloride (mp 82-83°) (Helv. Chim. Acta 1958, 41, 491). Alternatively, recrystallize in the cold from n-hexane/ethyl acetate.)

HPLC VARIABLES
Column: 250 × 4 5 μm Grom-Sil 120 ODS-3 CP porous encapsulated polymer-coated spherical particles (Grom, Herrenberg, Germany)
Mobile phase: Gradient. A was MeCN:THF:100 mM pH 6.7 sodium acetate buffer 20:2:78. B was MeCN:THF 98:2. A:B from 100:0 to 95:5 over 15 min, to 85:15 over 18 min, to 75:25 over 24 min, to 50:50 over 10 min, to 0:100 over 1 min, maintain at 0:100 for 7 min, return to initial conditions over 0.1 min, re-equilibrate for 10 min.
Column temperature: 45
Flow rate: 1 for 5 min, to 1.25 over 0.1 min, maintain at 1.25
Injection volume: 20
Detector: UV 320

CHROMATOGRAM
Retention time: 7.75 (Asp), 8.95 (Glu), 18.05 (Ser), 19.56 (Arg), 19.94 (Gly), 20.84 (Thr), 21.45 (Pro), 22.37 (Ala), 26.77 (Val), 29.83 (Tyr), 31.21 (Met), 34.65 (Ile), 35.43 (Leu), 38.11 (Phe), 63.99 (His), 64.42 (Lys)
Limit of detection: 0.5 pmole

KEY WORDS
derivatization

REFERENCE
Kirschbaum,J.; Brückner,H. Amino acid analysis by derivatization with chromogenic 4-phenylazobenzyloxycarbonyl chloride (PAZ-Cl): Comparison of reversed phases, *Chromatographia*, **1996**, *43*, 275–278.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL Amino acid solution in 250 mM pH 8.8 borate buffer + 10 μL 4 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 10 μL reagent, mix, let stand for 3.5 min, add 10 mL (?) MeCN:glacial acetic acid 80:20, mix, inject an aliquot. (Reagent was 150 mL 500 mM hydroxylamine hydrochloride containing 340 μL 850 mM NaOH and 10 μL 2-(methylthio)ethanol.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-Hypersil
Mobile phase: Gradient. A was MeOH:water 15:85 containing 30 mM $(NH_4)H_2PO_4$, pH 6.5. B was MeOH:water 15:85. C was MeCN:water 90:10. A:B:C 17:68:15 for 1 min, to 43.2:46:12 over 31 min, to 0:0:100 over 0.05 min, maintain at 0:0:100 for 1.95 min.
Column temperature: 38
Flow rate: 1
Detector: F ex 270 em 316

CHROMATOGRAM
Retention time: 3 (aspartic acid), 3.5 (glutamic acid), 7.5 (hydroxyproline), 9.7 (serine), 10.3 (histidine), 10.6 (glycine), 11.2 (threonine), 11.8 (alanine), 12.5 (proline), 13.5 (tyrosine), 15 (arginine), 16 (valine), 16.5 (methionine), 18.5 (isoleucine), 19 (leucine), 20 (phenylalanine)

KEY WORDS
derivatization

REFERENCE
Ou,K.; Wilkins,M.R.; Yan,J.X.; Gooley,A.A.; Fung,Y.; Sheumack,D.; Williams,K.L. Improved high-performance liquid chromatography of amino acids derivatized with 9-fluorenylmethyl chloroformate, *J.Chromatogr.A*, **1996**, *723*, 219–225.

SAMPLE
Matrix: solutions
Sample preparation: Rapidly mix 1 mL 10 mM dansyl chloride in MeCN with 2 mL amino acid solution in buffer, sonicate for 10 min in the dark, let stand at room temperature in the dark for 40 min, inject a 25 μL aliquot. (Buffer was 40 mM lithium carbonate adjusted to pH 9.5 with HCl.)

HPLC VARIABLES
Guard column: 70 × 2 Partisil ODS
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 17:83 containing 0.5 mM tris(2,2'-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$)

Flow rate: 1
Injection volume: 25
Detector: chemiluminescence following post-column electrochemical oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$. The column effluent flowed through a Bioanalytical Systems BAS 100A electrochemical detector with a Pt electrode at 1250 mV (relative to an Ag/AgCl reference electrode) to the chemiluminescence detector.

CHROMATOGRAM

Retention time: 13 (Glu), 23 (Asn), 32 (Ser), 40 (Thr), 46 (Gly), 52 (Ala)
Limit of detection: 100 nM

KEY WORDS

derivatization

REFERENCE

Skotty,D.R.; Lee,W.-Y.; Nieman,T.A. Determination of dansyl amino acids and oxalate by HPLC with electrogenerated chemiluminescence detection using tris(2,2'-bipyridyl)ruthenium(II) in the mobile phase, *Anal.Chem.*, **1996**, *68*, 1530–1535.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate 25 μL of an amino acid solution to dryness under a stream of nitrogen at 50°, add MeCN, evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL MeCN:MeOH:triethylamine 10:5:2, add 3 μL butylisothiocyanate, sonicate for 1 min, heat at 40° for 30 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 20 mM ammonium acetate, filter (0.25 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid. B was MeCN:MeOH:THF:20 mM Na_2HPO_4 50:2.5:0.75:46.75. C was MeCN:water 70:30. A:B:C from 100:0:0 to 85:15:0 over 8 min, to 70:20:10 over 6 min, to 60:20:20 over 6 min, to 30:20:50 over 5 min, to 10:20:70 over 5 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 20 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 8 (Asp), 9 (Glu), 12 (Hyp), 14 (Asn, Ser), 14.3 (Gly), 14.6 (Gln), 15.6 (His), 15.9 (Thr), 16.2 (Ala), 16.6 (Arg), 17 (Pro), 20.3 (Tyr), 20.6 (Val), 22 (Met), 23 (Ile), 23.5 (Leu), 24 (cystine), 25.3 (Phe), 26 (Trp), 26.5 (Lys), 27.7 (cysteine)
Internal standard: norleucine (24.5)
Limit of detection: 3.9 pmole

KEY WORDS

derivatization; comparison with phenylisothiocyanate derivatization

REFERENCE

Woo,K.L.; Hwang,Q.C.; Kim,H.S. Determination of amino acids in the foods by reversed-phase high-performance liquid chromatography with a new precolumn derivative, butylthiocarbamyl amino acid, compared to the conventional phenylthiocarbamyl derivatives and ion-exchange chromatography, *J.Chromatogr.A*, **1996**, *740*, 31–40.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 50 mM aqueous solution with 20 μL 1 M sodium bicarbonate, add 100 μL 1% Marfey's reagent in acetone, vortex, heat at 37° for 1 h, add 20 μL 1 M HCl, add 810 μL MeCN, inject a 1 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-AR (Nacalai Tesque)

Mobile phase: Gradient. MeCN:buffer from 15:85 to 45:55 over 5 min, re-equilibrate at initial conditions for 15 min. (Buffer was 100 mM ammonium acetate in water, adjusted to pH 3 with trifluoroacetic acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 1

Detector: UV 340

CHROMATOGRAM

Retention time: 3.7 (D-β-threo-hydroxyaspartic acid), 3.8 (L-β-threo-hydroxyaspartic acid), 6.0 (D-histidine (mono-derivative)), 6.1 (D-β-erythro-hydroxyaspartic acid), 6.7 (L-asparagine), 6.7 (L-histidine (mono-derivative)), 6.9 (L-β-erythro-hydroxyaspartic acid), 7.3 (D-asparagine), 8.2 (L-aspartic acid), 8.5 (D-ornithine (mono-derivative)), 8.8 (L-glutamine), 9.0 (L-homoserine), 9.2 (L-serine), 9.3 (L-arginine), 9.4 (L-ornithine (mono-derivative)), 9.6 (D-glutamine), 9.6 (D-arginine), 9.8 (D-aspartic acid), 9.9 (D-serine), 10.0 (D-citrulline), 10.4 (L-allo-threonine), 10.4 (L-threonine), 10.5 (D-homoserine), 10.6 (L-lysine (mono-derivative)), 10.7 (L-citrulline), 10.8 (D-lysine (mono-derivative)), 10.8 (L-glutamic acid), 12.3 (D-allo-threonine), 12.3 (L-O-methylserine), 13.1 (D-glutamic acid), 14.8 (D-threonine), 14.8 (L-alanine), 15.7 (L-proline), 17.2 (D-O-methylserine), 17.9 (L-2-amino-n-butyric acid), 18.2 (D-proline), 19.1 (D-alanine), 20.2 (L-methionine), 21.9 (L-histidine (bis-derivative)), 23.2 (L-valine), 23.8 (D-2-amino-n-butyric acid), 24.0 (L-norvaline), 25.3 (D-histidine (bis-derivative)), 26.4 (D-methionine), 27.7 (L-phenylalanine), 28.7 (L-isoleucine), 29.6 (L-leucine), 29.7 (D-valine), 29.9 (L-norleucine), 30.4(D-norvaline), 31.4 (L-ornithine (bis-derivative)), 31.4 (L-lysine (bis-derivative)), 33.2 (D-phenylalanine), 34.5 (D-lysine (bis-derivative)), 34.5 (D-ornithine (bis-derivative)), 35.3 (D-isoleucine), 35.9 (D-leucine), 36.5 (D-norleucine), 37.8 (L-tyrosine (bis-derivative)), 42.6 (D-tyrosine (bis-derivative))

KEY WORDS

derivatization; chiral

REFERENCE

Fujii,K.; Ikai,Y.; Mayumi,T.; Oka,H.; Suzuki,M.; Harada,K.-I. A nonempirical method using LC/MS for determination of the absolute configuration of constituent amino acids in a peptide: Elucidation of limitations of Marfey's method and of its separation mechanism, *Anal.Chem.*, **1997**, *69*, 3346–3352.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL aliquot of a 10-25 μg/mL solution of amino acids in water with 100 μL buffer, add 50 μL 5% 2,4-dinitrofluorobenzene in MeCN, vortex, heat at 50° for 1 h, add 250 μL 1% acetic acid, add 500 μL MeCN:water 50:50, inject a 20 μL aliquot. (Buffer was 100 mM sodium bicarbonate adjusted to pH 9.5 with 100 mM sodium carbonate.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm 100 Å Kromasil (EKA Nobel) derivatized with a chiral quinine carbamate (J. Chromatogr.A 1996, 741, 33)

Mobile phase: MeCN:THF:110 mM ammonium acetate 44.55:0.45:55, adjusted to pH 5.5 with glacial acetic acid

Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 390

CHROMATOGRAM

Retention time: 13.11 (L-Thr), 15.64 (L-Pro), 18.29 (D-Thr), 18.90 (L-Ala), 19.93 (L-Val), 21.47 (D-Pro), 22.43 (D-Ala), 23.79 (L-Leu), 26.04 (D-Val), 30.37 (D-Leu), 33.47 (L-Phe), 40.93 (D-Phe), 48.00 (L-Trp), 66.03 (D-Trp)

KEY WORDS

derivatization; chiral

REFERENCE

Lammerhofer,M.; Di Eugenio,P.; Molnar,I.; Lindner,W. Computerized optimization of the high-performance liquid chromatographic enantioseparation of a mixture of 4-dinitrophenyl amino acids on a quinine carbamate-type chiral stationary phase using DRYLAB, *J.Chromatogr.B*, **1997**, *689*, 123–135.

SAMPLE

Matrix: soybean meal
Sample preparation: Mix 200 mg soybean meal with 15 mL 0.1% phenol in 6 M HCl, pump out the tube then flush with nitrogen 3 times, heat at 145° for 4 h, filter, evaporate to dryness under reduced pressure at 50°, reconstitute with 50 mL 10 mM HCl. Add a 5 mL aliquot to a 100 × 13 Dowex 5X8 cation exchange column, elute with 4 M ammonia. Evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 50 mL 10 mM HCl. Evaporate a 500 μL aliquot to dryness under a stream of nitrogen at 50°, add 30 μL MeCN, evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL 2.5 mM L-norleucine in MeCN:MeOH:triethylamine 10:5:2, add 3 μL benzylisothiocyanate, heat at 50° for 30 min, evaporate to dryness under a stream of nitrogen with simultaneous vacuum pump evacuation at room temperature, reconstitute the residue in 50 μL MeCN, evaporate to dryness as before, reconstitute the residue in 1 mL initial mobile phase, filter (0.2 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeOH:THF:20 mM NaH_2PO_4 5:1.5:93.5, adjusted to pH 6.8 with phosphoric acid. B was MeCN:MeOH:THF:20 mM NaH_2PO_4 50:2.5:0.75:46.75, adjusted to pH 6.8 with phosphoric acid. C was MeCN:water 70:30. A:B:C from 100:0:0 to 80:20:0 over 10 min, to 76:20:4 over 5 min, to 70:20:10 over 5 min, to 50:30:20 over 10 min, to 30:35:35 over 10 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 20 min.
Column temperature: 40
Flow rate: 1.2
Injection volume: 10
Detector: UV 246

CHROMATOGRAM

Retention time: 4 (Asp), 4.5 (Glu), 7 (Hyp), 9.5 (Asn), 10 (Gln), 10.5 (Ser), 11 (Gly), 11.5 (His), 12 (Pro), 12.5 (Arg), 13 (Thr), 13.5 (Ala), 17.5 (ammonia), 19 (Asp), 20 (Val), 20.5 (Tyr), 22 (Met), 25 (Ile), 25.5 (Leu), 27.5 (cystine), 28 (Phe), 29.5 (Trp), 32 (Lys), 34.5 (cysteine)
Internal standard: L-norleucine (26)
Limit of detection: 3.9 pmole

KEY WORDS

derivatization; SPE; comparison with phenylisothiocyanate derivatization

REFERENCE

Woo,K.-L.; Ahan,Y.-K. Determination of protein amino acids as benzylthiocarbamyl derivatives compared with phenylthiocarbamyl derivatives by reversed-phase high-performance liquid chromatography, ultraviolet detection and precolumn derivatization, *J.Chromatogr.A*, **1996**, *740*, 41–50.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: MeCN:water:phosphoric acid 13:87:0.15

Flow rate: 1

Injection volume: 4

Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.61 (cysteic acid), k' 0.93 (glutathione), k' 0.98 (ethanolamine), k' 1.24 (asparagine), k' 1.26 (taurine), k' 1.30 (methionine), k' 1.71 (ammonia), k' 1.58 (glutamine), k' 1.94 (cystathionine), k' 1.95 (leucine), k' 1.95 (lysine), k' 1.96 (isoleucine), k' 2.01 (cysteine), k' 2.14 (proline), k' 2.43 (homocarnosine), k' 2.45 (urea), k' 2.54 (arginine), k' 2.54 (hydroxyproline), k' 2.70 (glutamic acid), k' 2.86 (aspartic acid), k' 2.86 (serine), k' 3.65 (threonine), k' 4.01 (glycine), k' 4.55 (norvaline), k' 5.91 (alanine), k' 5.93 (valine), k' 6.48 (GABA), k' 8.58 (6-aminocaproic acid), k' 11.42 (α-aminobutyric acid), k' 16.22 (tryptophan), k' 22.13 (tyrosine)

Internal standard: β-aminoisobutyric acid (k' 9.25)

Limit of quantitation: 10 pmole

KEY WORDS

rat; brain; derivatization

REFERENCE

Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize mouse liver with chloroform:MeOH 2:1, extract the homogenate with water. Lyophilize the aqueous phase, reconstitute with water. Remove a 100 μL aliquot, add 10 μL 10 mM NaOH, add 100 μL 100 mM pH 9.0 borate buffer, add 100 μL 1 mM dansyl chloride in MeCN, vortex, heat at 40° for 45 min, cool to room temperature, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChrosorb RP-18

Mobile phase: Gradient. A was MeCN:10 mM pH 7.0 phosphate buffer 10:90. B was MeCN:10 mM pH 7.0 phosphate buffer 50:50. A:B from 75:25 to 30:70 over 20 min, maintain at 30:70 for 10 min, return to initial conditions over 0.1 min, re-equilibrate for 10 min.

Flow rate: 3

Injection volume: 75

Detector: F ex 330 em 565, UV 254

CHROMATOGRAM
Retention time: 8.24 (tyrosine), 11.9 (tryptophan)

KEY WORDS
derivatization; mouse; liver

REFERENCE
Manwaring,J.D.; Csallany,A.S. Identification of vitamin E-dependent water soluble fluorescent compounds in mouse tissues, *Lipids*, **1990**, *25*, 22–26.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass/PTFE homogenizer) tissue in 320 mM sucrose, mix 60 μL homogenate with 100 μL 400 mM perchloric acid, centrifuge at 10000 g for 10 min. Remove a 100 μL aliquot of the supernatant and add it to 65 μL 2 M potassium bicarbonate, centrifuge at 10000 g for 10 min. Remove a 10 μL aliquot of the supernatant and mix it with 10 μL 1 mM α-aminoadipic acid, 20 μL 25 mM sodium bicarbonate, and 80 μL 4 mM dabsyl chloride in MeCN, vortex thoroughly, heat at 70° for 12 min, add 380 μL 20% acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher C18
Mobile phase: Gradient. A was DMF:25 mM pH 6.4 sodium acetate buffer 4:96. B was MeCN. A:B 85:15 for 2 min, to 70:30 over 28 min, to 59:41 over 5 min, to 46:54 over 2 min, to 43:57 over 2 min, to 33:67 over 2 min, to 10:90 over 1 min, return to initial conditions over 2 min, re-equilibrate for 11 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 436

CHROMATOGRAM
Retention time: 10 (aspartate), 11 (glutamate), 19 (glutamine), 20 (serine), 21 (threonine), 22 (glycine), 23 (alanine), 25 (arginine, taurine), 26.5 (proline, gamma-aminobutyric acid), 27.5 (valine), 30 (methionine), 31.5 (isoleucine) 32.5 (leucine), 35 (phenylalanine), 37.5 (cystine), 38.5 (ammonia), 40.5 (lysine), 41 (histidine), 42 (tyrosine)
Internal standard: α-aminoadipic acid (12)

KEY WORDS
derivatization; brain

REFERENCE
Watanabe,A.; Semba,J.; Kurumaji,A.; Kumashiro,S.; Toru,M. Measurement of glutamate, aspartate and glycine and its potential precursors in human brain using high-performance liquid chromatography by pre-column derivatization with dimethylaminoazobenzene sulphonyl chloride, *J.Chromatogr.*, **1992**, *583*, 241–245.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize oocytes with 3 volumes of water, add 9 volumes ice-cold MeOH, centrifuge at 4° at 2000 g for 10 min. Evaporate the supernatant to dryness under reduced pressure, resuspend in 100 μL MeOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 1-10 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10 per oocyte, let stand at room temperature for 15 min, dry under vacuum, reconstitute with 50 mM pH 6.8 ammonium acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 100 × 5 Nova-Pak C18
Mobile phase: Gradient. A was 50 mM pH 6.8 ammonium acetate buffer. B was MeCN: 100 mM ammonium acetate 50:50. A:B 100:0 for 5 min, to 90:10 over 1 min, maintain at 90:10 for 4 min, to 50:50 over 15 min, maintain at 50:50 for 5 min.
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6 (Asp), 3.5 (Glu), 9 (Ser), 10 (Gly), 12.5 (Asn), 13.3 (Gln), 15 (Thr), 15.4 (Ala, His), 17 (Pro), 20.6 (Tyr), 20.7 (Val), 21.7 (Met), 23 (Ile), 23.3 (Leu), 25 (Phe), 26 (Trp), 27.5 (Lys)

KEY WORDS
derivatization; oocytes

REFERENCE
O'Connor,C.M. Analysis of aspartic acid and asparagine metabolism in *Xenopus laevis* oocytes using a simple and sensitive HPLC method, *Mol.Reprod.Dev.*, **1994**, *39*, 392–396.

SAMPLE
Matrix: tissue culture
Sample preparation: Dilute tissue culture 50-fold with initial mobile phase, filter (0.45 μm), remove a 10 μL aliquot of the filtrate and add it to 20 μL reagent, mix, inject. (Prepare reagent by mixing 12 mL Fluoraldehyde (Pierce) with 10 μL mercaptoethanol. Fluoraldehyde contains o-phthalaldehyde, mercaptoethanol, and Brij-35.)

HPLC VARIABLES
Guard column: C18
Column: Resolve C18 (Waters)
Mobile phase: Gradient. A was MeOH:THF:50 mM pH 7.5 sodium acetate buffer containing 50 mM sodium phosphate 2:2:96. B was MeOH:water 65:35. A:B from 100:0 to 0:100 over 47 min, maintain at 0:100 for 15 min, re-equilibrate at initial conditions for 30 min.
Flow rate: 1.5
Detector: F ex 334 (filter) em 425 (filter)

CHROMATOGRAM
Retention time: 4.6 (Asp), 8.3 (Glu), 17.6 (Ser), 20.3 (Gln), 20.5 (His), 23.3 (Gly), 24.6 (Thr), 26.0 (Arg), 29.5 (Ala), 32.9 (Tyr), 40.0 (Met), 40.5 (Val), 42.5 (Phe), 44.7 (Ile), 45.8 (Leu), 52.0 (Lys)

KEY WORDS
derivatization; paper contains discussion of ways to increase column life

REFERENCE
Krok,K.A.; Seaver,S.S. Realities of automating OPA HPLC amino acid analyses, *BioTechniques*, **1991**, *10*, 664–670.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize eyes or nervous tissue with a 9-fold excess of 8% perchloric acid, centrifuge at 0° at 20000 g for 10 min. Neutralize the supernatant with solid potassium bicarbonate, centrifuge. Mix a 100 μL aliquot of the supernatant with 400 μL 300 mM pH 11.0 borate/NaOH buffer and 500 μL 18 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand at room temperature for 45 s, wash twice with 2 mL portions of pentane, filter (0.45 μm) the aqueous phase, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 4.6 Nucleosil C18

Column: 250 × 4.6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: Gradient. A was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 10:75:15, adjusted to pH 2.0. B was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 20:10:70, adjusted to pH 5.3. C was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 60:10:30, adjusted to pH 6.2. A:B:C from 100:0:0 to 15:85:0 over 3 min, to 13:87:0 over 12 min, to 0:30:70 over 70 min, to 0:0:100 over 0.1 min, maintain at 0:0:100 for 9.9 min. (Adjust pH with 6 M HCl or 6 M NaOH.)
Column temperature: 55
Flow rate: 0.7
Injection volume: 20
Detector: F ex 260 em 310

CHROMATOGRAM

Retention time: 18.5 (D-Arg), 19.5 (L-Arg), 22.5 (taurine), 25.4 (D-Asn), 26 (L-Asn), 26.5 (D-Gln), 27.5 (L-Gln), 29 (D-citrulline), 29.3 (L-citrulline), 31.5 (D-Hyp, L-Ser, D-Ser), 32 (L-Hyp), 32.5 (D-Asp), 33.5 (L-Asp), 36 (D-Glu), 36.5 (L-Glu), 37 (D-Thr), 37.5 (L-Thr), 38.5 (Gly), 41.5 (L-α-aminoadipic acid), 45 (L-Pro), 48 (D-Ala, L-Ala), 50.5 (L-Pro, D-Pro), 52 (gamma-aminobutyric acid), 55 (D-β-aminobutyric acid, L-β-aminobutyric acid, L-α-aminobutyric acid), 57 (D-Met), 58 (L-Met), 62.5 (D-Val), 63.5 (L-Val), 65.5 (D-Phe), 66.5 (L-Phe), 71 (D-Ile), 71.5 (D-Leu), 72 (L-Ile), 72.5 (L-Leu), 75 (D-cystine), 76.5 (cystathionine), 77 (L-cystine), 80.5 (D-Hyl, L-Hyl), 88.5 (D-ornithine), 90 (L-ornithine), 91.5 (D-Lys), 92.5 (L-Lys)
Limit of detection: <1 pmole

KEY WORDS

derivatization; chiral; compounds which co-elute under these conditions can be resolved by altering mobile phase conditions.; crab; lobster; prawn; crayfish; nervous tissue; eye

REFERENCE

Okuma,E.; Abe,H. Simultaneous determination of D- and L-amino acids in the nervous tissues of crustaceans using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *660*, 243–250.

SAMPLE

Matrix: urine
Sample preparation: Freeze urine at -20°, thaw, stir thoroughly, allow to settle. 600 μL Supernatant + 600 μL concentrated HCl, heat at 110 ± 5° for 18 h, cool, filter, dilute (if necessary). Evaporate a 25-200 μL aliquot to dryness under reduced pressure, reconstitute with 30 μL water:EtOH:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 50 μL phenyl isothiocyanate:EtOH:triethylamine:water 10:70:10:10, vortex, let stand for 10 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, filter, inject an aliquot. (Buffer was MeCN:10 mM NaH_2PO_4 containing 2 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) 1.8:99.2, pH 6.0.)

HPLC VARIABLES

Guard column: Guard-Pak (Waters)
Column: 250 × 4 5 μm octadecyl (IBM)
Mobile phase: Gradient. A was MeCN:10 mM NaH_2PO_4 containing 2 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) 1.8:99.2, pH 6.0. B was MeCN:water 60:40. A:B 100:0 for 7 min, to 0:100 over 1 min, maintain at 0:100 for 2 min, return to initial conditions over 1 min, re-equilibrate for 7 min
Column temperature: 34 or 43
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Retention time: 2.60 (O-phosphoserine), 2.77 (aspartic acid), 3.12 (glutamic acid), 4.13 (α-aminoadipic acid), 5.10 (hydroxyproline), 6.22 (phosphoethanolamine), 6.74 (asparagine), 6.90 (serine), 7.67 (glycine), 7.75 (glutamine), 8.5 (homoserine), 8.72 (sararcosine), 9.24 (β-alanine), 9.25 (anserine), 9.54 (glycerophosphoryl ethanolamine), 10.57 (taurine), 11.57 (citrulline), 12.50 (threonine), 12.84 (gamma-aminobutyric acid), 13.53 (alanine), 14.29 (β-aminoisobutyric acid), 15.06 (histidine), 16.31 (proline), 17.47 (carnosine)

Limit of quantitation: 250 pmoles

KEY WORDS

derivatization

REFERENCE

Lippincott,S.; Chesney,R.W.; Friedman,A.; Pityer,R.; Barden,H.; Mazess,R.B. Rapid determination of total hydroxyproline (HYP) in human urine by HPLC analysis of the phenylisothiocyonate (PITC)-derivative, *Bone*, **1989**, *10*, 265–268.

SAMPLE

Matrix: urine

Sample preparation: 80 μL Urine + 20 μL 10 mM N-methylalanine containing 1 mM norvaline, mix, filter (Ultrafree-MC) while centrifuging at 5000 g for 30 min. Remove a 6 μL aliquot of the ultrafiltrate and add it to 5 μL 0.5% 3-mercaptopropionic acid in 1 M pH 10.4 borate buffer, mix, add 1.5 μL 120 mM iodoacetic acid in 140 mM NaOH, mix, add 5 μL reagent, mix, add 2 μL 9-fluorenylmethyl chloroformate, mix, add 2.5 μL 1 M acetic acid, mix, inject the whole amount. (Reagent was 20 mg/mL o-phthalaldehyde in MeOH:500 mM pH 10.4 borate buffer:3-mercaptopropionic acid 10:88:2.)

HPLC VARIABLES

Guard column: 20 × 4 5 μm ODS Hypersil

Column: 300 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 60 mM pH 6.86 sodium acetate buffer containing 0.044% triethylamine. B was MeCN:MeOH:100 mM pH 5.45 sodium acetate buffer 74.5:4.5:21. A:B from 100:0 to 94.4:5.6 over 1 min, to 93.8:6.2 over 6 min, maintain at 93.8:6.2 for 2 min, to 92.3:7.7 over 12 min, maintain at 92.3:7.7 for 7 min, to 92:8 over 7 min, to 90.8:9.2 over 4 min, to 90.5:9.5 over 3 min, to 84:16 over 6 min, maintain at 84:16 for 1 min, to 82:18 over 1 min, to 78:22 over 20 min, to 72:28 over 7 min, to 68:32 over 8 min, to 0:100 over 9 min, maintain at 0:100 for 5 min, return to initial conditions over 1 min.

Column temperature: 40

Flow rate: 0.8

Injection volume: 22

Detector: F ex 340 em 450 for 79.5 min, F ex 260 em 315 for 1.5 min, F ex 340 em 450 for 6 min, F ex 260 em 315 for 13 min

CHROMATOGRAM

Retention time: 5.5 (Asp), 6.5 (Glu), 8 (Cys (S-carboxymethylated)), 9 (Aad), 10 (Asn), 11 (Ser), 11.5 (Homocysteine (S-carboxymethylated)), 15 (Gln), 17.5 (His), 18 (Gly), 20 (Thr), 25 (Cit), 26 (1-Methylhistidine), 28 (β-Alanine), 29.5 (Arg), 30.5 (3-Methylhistidine), 33 (Ala), 35 (Tau), 38.5 (Ans), 39 (Car), 40 (β-aminoisobutyric acid), 41 (gamma-aminobutyric acid), 52 (Tyr), 52.5 (Abu), 57 (Etn), 60 (Val), 61.5 (Met), 65 (Cysta), 70 (Trp), 71.5 (Ile), 72.5 (Phe), 75 (Hyl), 76.5 (Leu), 80 (Hyp), 83.5 (Lys), 85 (Orn), 90 (Sar), 91 (Pro)

Internal standard: norvaline (62.5), N-methylalanine (91.5)

Limit of detection: 50-500 fmole

Limit of quantitation: 10 μM

KEY WORDS

derivatization; ultrafiltrate

REFERENCE
Carducci,C.; Birarelli,M.; Leuzzi,V.; Santagata,G.; Serafini,P.; Antonozzi,I. Automated method for the measurement of amino acids in urine by high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *729*, 173–180.

SAMPLE
Matrix: vegetables
Sample preparation: Extract 100 g potato tubers with 100 mL boiling water for 2 h, centrifuge at 5000 g for 10 min, filter (0.45 μm) the supernatant, dilute 100 μL of the filtrate with 900 μL 111 ng/mL norvaline in water, add a 1 μL aliquot to 5 μL 0.4 N pH 10.4 potassium borate buffer and 1 μL reagent, mix, add 1 μL 2.5 mg/mL 9-fluorenylmethyl chloroformate in anhydrous MeCN, mix, inject the whole amount. (Prepare reagent by dissolving 10 mg o-phthalaldehyde in 100 μL MeOH, make up to 1 mL with 0.4 N pH 10.4 borate buffer, add 20 μL 3-mercaptopropionic acid. Derivatization was performed automatically and took 5 min. o-Phthalaldehyde derivatized primary amino acids and 9-fluorenylmethyl chloroformate derivatized secondary amino acids (proline and hydroxyproline).)

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4 3 μm Hypersil ODS
Mobile phase: Gradient. A was THF:18 mM sodium acetate containing 0.02% triethylamine (adjusted to pH 7.2 with 1% acetic acid) 0.3:99.7. B was MeCN:MeOH:100 mM pH 7.2 sodium acetate 40:40:20. A:B from 100:0 to 94:6 over 0.5 min, to 80:20 over 2.5 min, to 50:50 over 3.5 min, to 25:75 over 1.5 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Column temperature: 40
Flow rate: 1.4
Injection volume: 8
Detector: F ex 340 em 450, after 6.8 min F ex 264 em 313

CHROMATOGRAM
Retention time: 0.709 (aspartic acid), 0.831 (glutamic acid), 1.747 (asparagine), 1.871 (serine), 2.160 (glutamine), 2.286 (histidine), 2.439 (glycine), 2.602 (threonine), 3.305 (alanine), 3.446 (arginine), 4.185 (tyrosine), 5.113 (valine), 5.221 (methionine), 5.657 (tryptophan), 5.835 (phenylalanine), 5.928 (isoleucine), 6.224 (leucine), 6.435 (lysine), 6.903 (hydroxyproline), 7.982 (proline)
Internal standard: norvaline (5.394)
Limit of quantitation: 20 pmole

KEY WORDS
derivatization; potato tubers

REFERENCE
Bartók,T.; Szalai,G.; Lorincz,Z.; Börcsök,G.; Sági,F. High-speed RP-HPLC/FL analysis of amino acids after automated two-step derivatization with o-phthaldialdehyde/3-mercaptopropionic acid and 9-fluorenylmethyl chloroformate, *J.Liq.Chromatogr.*, **1994**, *17*, 4391–4403.

SAMPLE
Matrix: yogurt
Sample preparation: 15 g Yogurt + 45 mL MeOH:water 80:20, stir for 10 min, centrifuge at 1630 g. Remove the supernatant and evaporate it to about 10 mL under reduced pressure, add 10 mL of a saturated solution of picric acid, mix, centrifuge at 1630 g. Remove the supernatant and wash it twice with 20 mL portions of light petroleum (bp 40-60°): diethyl ether 50:50, add the aqueous phase to a 50 × 10 column of Dowex 50W-X8 cation-exchange resin, wash with water, elute with 30 mL 2 M aqueous ammonia, evaporate the eluate to dryness, reconstitute with 500 μL 133 mM pH 10.4 borate buffer. Remove a 4

μL aliquot and add it to 2 μL 5 mg/mL o-phthaldialdehyde in 133 mM pH 10.4 borate buffer, add 2 μL 8 mg/mL N-acetyl-L-cysteine in 133 mM pH 10.4 borate buffer, mix for 3 min, inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. A was 40 mM pH 6.5 sodium acetate. B was MeCN. A:B from 100: 0 to 82:18 over 1 h.
Column temperature: 26
Flow rate: 0.9
Detector: UV 338

CHROMATOGRAM

Retention time: 3.8 (D-Asp), 4.1 (L-Asp), 7.4 (L-Glu), 8.0 (D-Glu), 12.3 (L-Ser), 12.7 (L-Asn), 12.9 (D-Ser), 13.7 (D-Asn), 16.5 (L-Gln), 18.0 (D-Gln), 18.2 (D,L-Thr), 18.4 (Gly), 21.0 (L-His), 21.6 (D-His), 25.4 (L-Ala), 26.0 (D-Ala), 27.2 (L-Arg), 28.8 (D-Arg), 36.5 (L-Tyr), 38.9 (D-Tyr), 39.5 (L-Val), 43.6 (D-Val), 44.1 (L-Met), 45.1 (D-Met), 48.8 (L-Ile), 51.5 (L-Trp), 52.6 (D-Ile), 53.2 (D-Trp), 53.6 (D-Phe), 54.1 (L-Phe), 55.1 (L-Leu), 55.7 (D-Leu), 59.3 (L-Lys), 59.7 (D-Lys)

KEY WORDS

derivatization; chiral; SPE; comparison with derivatization procedures using other thiols

REFERENCE

Brückner,H.; Wittner,R.; Godel,H. Automated enantioseparation of amino acids by derivatization with *o*-phthaldialdehyde and N-acylated cysteines, *J.Chromatogr.*, **1989**, *476*, 73–82.